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Activities of the Four Optical Isomers of 2′,3′-Dideoxy-3′-Thiacytidine (BCH-189) against Human Immunodeficiency Virus Type 1 in Human Lymphocytes

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Four different isomers of 2′,3′-dideoxy-3′-thiacytidine [β-DL-(±)-BCH-189] were evaluated in primary human lymphocytes infected with human immunodeficiency virus type 1. The β-L-(−) isomer was the most potent enantiomer, with a median effective concentration of 1.8 nM and no discernible cytotoxicity up to 100 μM. The relative order of potencies for the isomers was β-L-(−) > β-DL-(±) racemic > β-D-(+) > α-L-(+) > α-D-(−). The β-L-(−) enantiomer was as potent as 3′-azido-3′-deoxythymidine.

The development of novel selective antiviral agents for the treatment of AIDS remains an important scientific and medical goal. Nucleoside analogs continue to provide a rich source of antiviral agents that can suppress or eliminate acute infection in lymphocytes and macrophages (18). To date, there are six nucleosides that are at various clinical states of use for the treatment of human immunodeficiency virus type 1 (HIV-1) infections in humans. These are 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxycytidine (DDC), 2′,3′-didehydro-3′-deoxy-thymidine (D4T), 3′-azido-2′,3′-dideoxyuridine (AzddU, CS-87, AZDU), and 3′-fluoro-3′-deoxythymidine (FLT) (18). A novel 2′,3′-dideoxynucleoside that is at an advanced preclinical stage of use is racemic 2′,3′-dideoxy-3′-thiacytidine [β-DL-(±)-BCH-189]. The structural difference between DDC and BCH-189 is the presence of a sulfur atom instead of the methylene group at the 3′ position of the furanose ring (Fig. 1). This compound was first described by Canadian scientists to be effective against HIV-1 in vitro (3, 29). More recently, Soudeyns et al. (26) reported that the median effective concentration (EC_{50}) of racemic BCH-189 is 0.73 μM in MT-2 cells. Those investigators (26) indicated that this compound is less toxic than AZT in these cells and reported no discernible cross-resistance between BCH-189- and AZT-resistant viruses. However, careful review of their data suggests that there is a slight increase in the EC_{50} between pairs of AZT-susceptible and -resistant isolates. The low toxicity of racemic BCH-189 was recently confirmed in human peripheral blood mononuclear (PBM) cell proliferation assays by using two different activation pathways (concanavalin A and CD3 monoclonal antibody) and natural killer cell cytotoxicity assays in K562 cells (10). It is of significance that our group, in collaboration with scientists at Yale University, reported that racemic BCH-189 is also effective against human hepatitis B virus in a human hepatoblastoma-derived cell line (cell line 2.2.15) that continuously synthesizes virus (8). The dual selective and potent inhibition of this compound against HIV and hepatitis B virus makes this class of compound particularly important (17).

BCH-189 has two chiral carbon atoms at the 1′ and 4′ positions on the oxathiolane ring (Fig. 1). Thus, a pair of diastereoisomers or four possible enantiomers can be present (the α and β diastereoisomers have the 1′ and 4′ substituents in *trans* and *cis* relationships, respectively). All natural nucleosides and most biologically active nucleoside analogs have the β-D configuration. Recently, Chu et al. (7) reported the synthesis of BCH-189, which yields two of the possible enantiomerically pure forms (α-D and β-D) of this compound, starting from D-mannose. When the synthesis is started from L-mannose or L-gulose, the corresponding L isomers are obtained (5a). Choi et al. (4) described an improved means of synthesizing racemic BCH-189 exclusively with the desired β configuration by condensing the 1,3-oxathiolane moiety with protected cytidine in the presence of stannic chloride. We have found that all these isomers of BCH-189 can also be resolved by chromatography by using chiral columns as well as by enzymological methods (Table 1) (24a). Chu et al. (7) described the anti-HIV activity of the α and β-D isomers of BCH-189 in human lymphocytes, which suggested that the β-L-(−) isomer may be more potent. The availability of the chemical and analytical methodologies to stereoselectively prepare or resolve the α and β-D or β-L-analogs allowed us to compare their anti-HIV activities and toxicities in human PBM, CEM, and Vero cells. Results of studies in thymidine kinase (TK)-deficient CEM cells (CEM-TK^-) and normal CEM cells infected with HIV with racemic and enantiomerically pure BCH-189 are provided. The effect of racemic BCH-189 against a pair of well-characterized AZT-resistant and -susceptible viruses in human PBM cells is also described.

The BCH-189 analogs described here were synthesized in our laboratories and were characterized by proton nuclear magnetic resonance by using techniques such as the nuclear Overhauser effect to confirm the assigned structure of the compound. The detailed synthesis of these compounds is reported elsewhere (4, 5a, 7). The optical rotations of the

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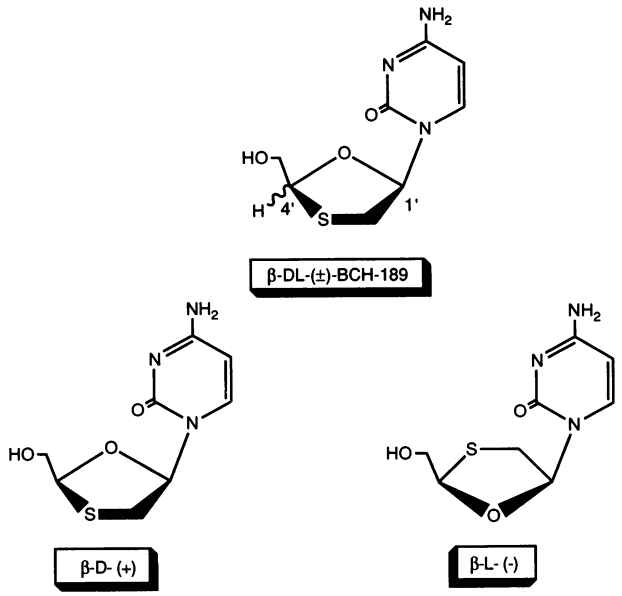

FIG. 1. Structures of racemic BCH-189 and the two most active antiviral forms.

compounds were determined by using a Jasco DIP-370 polarimeter with fresh solutions of the compounds in methanol. The optical rotations are given in Table 1.

The procedure used for the antiviral and cytotoxicity assays in human PBM cells has been described previously (24, 25), except that recombinant interleukin 2, which was obtained from Cetus Corp. (Emeryville, Calif.), was used. For these assays the LAV strain of HIV-1 was used. AZT-resistant and -susceptible virus strains 9F (G910-6) and 10 (H112-2) were obtained from D. Richman (Veterans Affairs, San Diego, Calif.) through the AIDS Research and Reference Program of the National Institutes of Health and were propagated in PBM cells as described previously (25). CEM (CEM-CCRF) cells are a T-lymphoblastoid cell line and were obtained from the American Type Culture Collection, Rockville, Md. CEM cells were maintained in RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml), as described previously (25). CEM-TK$^-$ cells were prepared in our laboratory by sequential passage of CEM cells in the presence of 5-bromo-2'-deoxyuridine. The lack of TK activity was determined as described previously (22). The viruses obtained from the PBM cell supernatant were titrated and stored in aliquots at −70°C until use. A 50% tissue culture infective dose of about 100 was used to infect all the cultures, and the viruses were harvested 6 days after infection. Cytotoxicity was determined in PBM and CEM cells on day 6 and in Vero cells on day 3 after treatment, as described previously (25). The EC_{50} and median inhibitory concentration (IC_{50}) were determined by the median effect method, which linearizes the dose-response data (5). Separation of the enantiomers was performed by using a cyclobond AC-I column obtained from Rannin Instruments (Woburn, Mass.). The conditions were as follows: isocratic 0.5% high-pressure liquid chromatographic (HPLC) grade methanol (J. T. Baker, Phillipsburg, N.J.) in water; flow rate, 1 ml/min; UV detection at 262 nm. The retention times obtained by use of these conditions are given in Table 1. The β-L-(−) and β-D-(+) enantiomers were resolved from racemic BCH-189 by this method. Fractions containing each of the pure enantiomers were pooled, frozen, and then lyophilized. The compounds were characterized by UV spectroscopy and by their retention times on HPLC in comparison with that of the other isomer. As anticipated, the L enantiomers have retention times lower than those of the D isomers (1). The concentration of the compounds was determined by UV spectroscopy. Stock solutions with known concentrations were prepared in water for biological evaluation.

TABLE 1. Retention times and optical rotations of pure isomers of BCH-189

BCH-189 isomer	Retention time (min)[a]	Optical rotation $[\alpha]_D^{25}$	Reference
β-L-(−)	10.5	−121.6 (c 1.1, MeOH[b])	
α-L-(+)	12.9	+146.6 (c 0.55, MeOH)	
β-D-(+)	11.4	+120.96 (c 1.04, MeOH)	7
α-D-(−)	13.3	−143.18 (c 0.62, MeOH)	7

[a] The HPLC conditions for separation by use of a chiral column are described in the text.
[b] MeOH, methanol.

Biological results obtained with the four isomers of BCH-189 compared with those obtained with the racemic compound, AZT, and AzddU are presented in Table 2. It is apparent that the β-L-(−) isomer was the most potent enantiomer, with an EC_{50} of 1.8 nM in PBM cells and no apparent cytotoxicity in PBM, CEM, or Vero cells when it was tested up to 100 μM. The order of increasing potency for the isomers was β-L-(−) > β-DL-(±) racemic > β-D-(+) > α-L-(+) > α-D-(−). The β-L-(−) enantiomer was of the same order of potency as AZT, whereas the β-D-(+) enantiomer was of the same order of potency as AzddU in this cell culture system. Unexpectedly the α-L-(+) enantiomer was shown to have a weak antiviral effect against HIV-1. No apparent toxicity was observed with the isomers or racemic BCH-189 in PBM and Vero cells at 100 μM. However, the β-D-(+) and racemic BCH-189 were toxic to CEM cells, with IC_{50}s of 2.7 and 52.6 μM, respectively.

In order to confirm that the antiviral activities of these synthetic compounds were associated with the correct isomer, racemic BCH-189 was chromatographed by using a chiral column, and the resolved β-L-(−) and β-D-(+) enantiomers were evaluated against HIV-1 in PBM cells. These compounds had retention times identical to those of the synthetic material obtained by using stereoselective synthesis. The concentrations of the material collected from the HPLC procedure were estimated from the UV absorbances of the compounds (based on λ_{max} = 280 nm and ε = 13,200 in water). The EC_{50} against HIV-1 in PBM cells for the β-L-(−) enantiomer was about 0.02 μM, whereas the EC_{50}s of racemic BCH-189 and the β-D-(+) enantiomer were 0.08 and 0.2 μM, respectively. In the same assay, the synthetic β-D-(+) enantiomer had an EC_{50} of 0.3 μM. The order of magnitude for the HPLC-resolved enantiomers against HIV-1 was the same as that obtained with the synthetic enantiomers (Table 2), providing additional evidence that the characterizations of the compounds were correct.

The compounds described in this report were also evaluated in CEM and CEM-TK$^-$ cells infected with HIV-1 (strain LAV). In general, the compounds that were found to be active in PBM cells were also considered to be active (EC_{50}, <1 μM) in CEM cells with approximately the same order of potency (Table 2). However, racemic BCH-189 was as potent as the β-L-(−) enantiomer in these cells. Interest-

TABLE 2. Antiviral activities and cytotoxicities of BCH-189 analogs in various cells[a]

BCH-189 isomer	Anti-HIV-1 activity (EC_{50} [μM]) in:			Cytotoxicity (IC_{50} [μM]) in:		
	PBM cells	CEM cells	CEM-TK$^-$ cells	PBM cells	CEM cells	Vero cells
β-DL-(±) racemic	0.06	0.07	0.09	>100	52.6	>100
β-L-(−)	0.002	0.07	0.02	>100	>100	>100
α-L-(+)	10.1	79.8	ND[b]	>100	>100	>100
β-D-(+)	0.2	0.1	0.08	>100	2.7	>100
α-D-(−)	>100	71.3	ND	>100	>100	>100
AZT	0.004	0.005	>1	>100	14.3	28.0
AzddU	0.2	0.01	>10	>100	>100	>100

[a] Values are means of duplicate or triplicate assays (using different donor cells for the PBM cells) derived from dose-response curves of five or more different concentrations. The correlation coefficient for each individual data set was ≥0.95. Variance from the mean was ±8% or less.
[b] ND, not determined.

ingly, in CEM-TK$^-$ cells, the two most potent enantiomers had EC_{50}s similar to those in normal CEM cells. However, AZT and AzddU, which are known to be phosphorylated by cellular TK (9), were active in CEM but not CEM-TK$^-$ cells. These results suggest that the BCH-189 isomers are probably phosphorylated by cytidine/deoxycytidine kinase rather than by TK. The possibility that one or both of the most active enantiomers are phosphorylated by 5′-nucleotidase has been ruled out (22a).

Using a pair of AZT-resistant and -susceptible viruses in human PBM cells, we were able to reproduce previously published results obtained with HeLa CD4$^+$ cells and AZT (14). As can be seen in Fig. 2, with AZT there was a 237-fold increased resistance between the two viruses. However, racemic BCH-189 was only sixfold more resistant to the pretherapy isolate than it was to the posttherapy AZT-resistant virus. This modest cross-resistance between AZT and racemic BCH-189 has been noted previously with a different AZT-resistant virus (26). The modest cross-resistance and the different initial phosphorylating enzyme used by BCH-189 suggest that this compound could be used in combination with AZT, provided that no synergistic toxicity is noted (23).

A number of reports have indicated that, for certain nucleoside analogs, only one enantiomeric form is active. For example, Vince et al. (28) demonstrated that carbovir, a carbocyclic guanosine analog, has anti-HIV-1 activity. Subsequently, this activity was found to be associated only with the (−) enantiomer (27). (±)-Dioxolane-T and β-D-(−)-dioxolane-T, compounds that are related to BCH-189, with an oxygen instead of a sulfur atom at the 3′ position, were reported to have moderate anti-HIV activities in lymphocytes (6, 19). As expected, the corresponding α isomer, α-D-(+)-dioxolane-T, did not exhibit any significant anti-

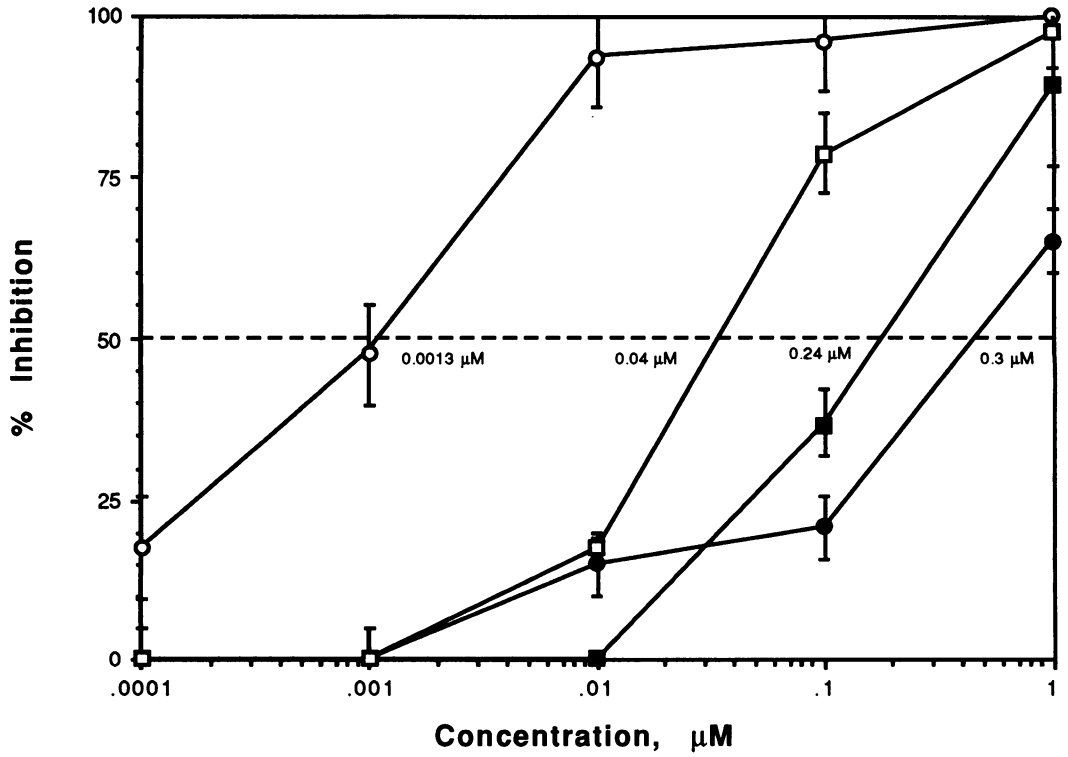

FIG. 2. Effect of racemic BCH-189 (squares) and AZT (circles) against AZT-resistant (solid symbols) and -susceptible (open symbols) HIV-1 isolates in human PBM cells. EC_{50}s were derived by the method of Chou and Talalay (5).

HIV activity (6). It is of interest that geometric isomers of DDC (β-L and α-D; EC_{50}s, <0.1 μM) had activity against HIV-1 in culture, whereas isomers of 2′,3′-dideoxyadenosine (DDA), D4C, and D4T had no significant activity (16). The investigators (6) were unable to confirm that the β-L-DDC and β-L-DDA analogs were inactive and active, respectively, against HIV-1, as reported by Okabe et al. (20) and Swedish workers (12a). However, the L isomer of AZT and related compounds (FLT, 3-deoxythymidine, D4T) had no antiviral effect (11). These compounds either cannot enter cells or are not substrates for cellular kinases. More recently, carbocyclic 3′-thia (A, T, C, G, and U analogs) and 3′-N-substituted nucleoside analogs related to BCH-189 have been synthesized, but they did not show any significant activity against HIV-1 (2, 13, 21). Another type of isomer that has been reported to have anti-HIV-1 activity in vitro is iso-DDA (12). The selective antiviral activity of enantiomers is not limited to nucleosides. For example, the (−) enantiomer of gossypol inhibited HIV-1 in PBM cells, whereas the (+) enantiomer was not active (15). In our studies, it is significant that both the β-L-(−) and β-D-(+) enantiomers of BCH-189, which are mirror images of each others, have significant antiviral activities at submicromolar concentrations and that one enantiomer is more toxic than the other in CEM cells (Table 2).

In summary, we demonstrated that the β-L-(−) enantiomer of BCH-189 is about 1 order of magnitude more potent in primary human lymphocytes than the β-D-(+) enantiomer is. The potent activity of the β-L-(−) enantiomer in these cells may be related to its resistance to cytidine/deoxycytidine deaminase, whereas the β-D-(+) isomer is a substrate for this enzyme (22a). The L-β-(−) enantiomer was also less toxic than racemic BCH-189 or the β-D-(+) enantiomer, suggesting that this isomer may have more favorable characteristics in vivo than racemic BCH-189. The unexpected finding that certain L isomers of nucleoside analogs of BCH-189 are potent and selective antiviral agents opens new approaches for the treatment of viral infections with nucleosides with the unusual L conformation.

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ADDENDUM IN PROOF

Since acceptance of this manuscript, an independent publication by Coates et al. (J. A. V. Coates, N. Cammack, H. J. Jenkinson, I. M. Mutton, B. A. Pearson, R. Storer, J. M. Cameron, and C. R. Penn, Antimicrob. Agents Chemother. **36**:202–205, 1992) has appeared with similar observations.

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